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THE APPLICATION OF ORTHOGONAL CONTRASTS TO DETERMINE HOMOGENEOUS GROUPS

ABSTRACT

The paper presents a modified approach to analysis of data obtained from experiments carried out according to classical factorial designs. Four examples were discussed in order to present details of proposed method. Modification of the analysis of variance presented here enables more effective use of information on how studied factors affect the means of dependent variable. The specificity of this approach is based on alternative multiple comparison procedure incorporating orthogonal contrasts to determine homogeneous groups.

Key words: experiment, data analysis, linear model, ANOVA, multiple comparisons, orthogonal contrasts

INTRODUCTION

From the nineteenth century, comparative experiments were frequently used in various fields of science. However, results of such experiments may be affected by errors if groups of experimental units are not equivalent at the start of the experiment. R. A. Fisher pointed out that if the experimental units (plots) are randomly assigned into groups, their equivalence should be guaranteed at least in terms of arithmetic means (Fisher, 1925, 1935; Cochran & Cox, 1957). Thus, his experimental designs provide both comparisons and randomization which eliminates also an unconscious bias of the experimenter. Random selection guarantees an impartiality towards each factor, even though its meaning is not known to the experimenter.

According to Fisher, consider the simplest randomized complete one-factor design, where each observation may be described by the following linear model:

$$y_{ij} = m + a_i + e_{ij} \text{ for } i = 1, \dots, p; j = 1, \dots, n_i; \sum_{i=1}^p n_i = n \quad (1)$$

where: y_{ij} – value of the dependent variable for the i -th level of factor A and the j -th replication; m – general mean; a_i – effect of i -th level of factor A; e_{ij} – experimental error for the i -th level of factor A and the j -th replication; p – number of levels of factor A; n_i – number of replications for i -th level of factor A; n – total number of observations. Assuming that $y_{ij} \sim N(m + a_i; \sigma^2)$ and $e_{ij} \sim N(0; \sigma^2)$.

The analysis of variance for such experimental data takes into account two types of variation: within object variation, arising from the variability (s_e^2) of the random deviations, and between object variation, arising from the variability (s_a^2) of tested effects a_i . The ratio of between-to-within object variation (called the F statistics) given as:

$$F_{emp} = \frac{s_a^2}{s_e^2} \quad (2)$$

has the F distribution under the null hypothesis written as:

$$H_0: \bigwedge_{1 \leq i \leq p} a_i = 0 \equiv H_0: \sum_{i=1}^p a_i^2 = 0. \quad (3)$$

If compared with the critical value of F distribution at certain significance level (α) the F statistics is the basis to confirm (if $F_{emp} \leq F_{\alpha; p-1; n-p}$) or deny (if $F_{emp} > F_{\alpha; p-1; n-p}$) the veracity of the null hypothesis given by formula (3) (Fisher, 1925; Cochran & Cox, 1957; Elandt, 1964; Searle, 1971; Wójcik & Laudański, 1989; Laudański, 1996; Mańkowski, 2002; Box et al., 2005; Montgomery, 2005).

Considering comparative experiments the most familiar procedure following rejection of null hypothesis (compared objects differ significantly) involves multiple comparisons in order to determine exactly which levels of given effect (factor) are equivalent in terms of analyzed response (usually mean value). Such procedures, called sometimes *post hoc tests* or *mean separation tests*, allow to extract specific subgroups of compared objects, homogeneous in terms of mean values

of the response, i.e. subgroups within objects which do not differ significantly between each other considering mean value of dependent variable. There are many methods of conducting multiple comparisons which differ with kind of comparisons they make (pairwise or with control) as well as with the type of error they control (individual or interval error rates). The list of mean separation tests includes a lot of procedures, like those of Tukey, Tukey-Kramer/Spjotvoll-Stoline or Student-Newman-Keuls, each based on the distribution of studentized range, Duncan – based on the distribution proposed by the author and individual error rates, Bonferroni – based on a modified usage of Student's t -distribution or Scheffé – based on the F distribution (Tukey, 1953; Dunnett, 1955; Cornifield & Tukey, 1956; Schéffe, 1959; Elandt, 1964; Duncan, 1975; Biegun & Gabriel, 1981; Hochberg & Tamhane, 1987; Hochberg, 1988; Wójcik & Laudański, 1989; Hsu & Nelson, 1998; Rafter et al., 2002). Moreover, multiple comparisons may be realized using the method of minimized within-group sum of squares (Wagner, 1977) and procedures derived from cluster analysis (Caliński & Corsten, 1985).

Probably the most versatile and frequently used for multiple comparisons is Tukey's procedure and its variants. It can be used to compare group means derived from orthogonal designs characterized by the same number of observations for each object, as well as from non-orthogonal ones characterized by an uneven number of observations for objects. For example, Student-Newman-Keuls or Duncan procedures should not be used for comparison of group means obtained from non-orthogonal designs as generally standard errors of mean differences may vary for each pair of compared object means.

If the null hypothesis is not rejected, there is no basis to conclude that objects are significantly different which means that all of them form one homogeneous group of means. Thus, the experimenter may sometimes fail to formally confirm a guess about diversity of tested objects.

In this paper we present an alternative multiple comparison procedure incorporating orthogonal contrasts to formally confirm an assumption about diversity of tested objects and to determine homogeneous groups.

The presented analyzes were performed in the IBM®SPSS program.

EXAMPLES AND DISCUSSION

Example 1

In a preliminary experiment 17 lines and 3 cultivars of rye has been studied. The unbalanced experiment was performed in 20 incomplete blocks, each split into plots of 10 m². Rye yield expressed in kilograms per plot was a dependent variable.

Analysis of variance for this experiment (Table 1) showed no differences between studied objects in terms of mean yield obtained from plot ($p = 0.2175$). The experimental accuracy for comparisons of mean yields calculated for analyzed objects (percentage ratio of standard deviation for object means to overall mean – coefficient of variation) ranged from 8.26% to 9.48%, whereas mean comparison accuracy was 8.92%. These values indicate that the experiment was carried out properly.

Considering Tukey procedure, mean yields for tested objects did not differ significantly (mean value of honestly significant difference HSD at $\alpha = 0.05$ was equal to 2.712, which means that mean comparison accuracy was 33.8%), whereas t -test showed significant difference between objects 16th and 9th (least significant difference LSD = $1.446 < 9.057 - 7.399 = 1.658$) at the significance level $\alpha = 0.05$ (mean comparison accuracy in this case, i.e. percentage ratio of LSD to overall mean, was 20.7%).

Splitting tested objects according to the results of the comparison by the Student's procedure into two subgroups and performing analysis of variance for such a dataset will be equivalent to performing analysis in a cross-hierarchical design: blocks \times objects within subgroups. Thus, it is possible to confirm the existence of differences between mean yields calculated for subgroups, even though variation of mean yields for objects within each subgroup is not significant.

Otherwise, if 20 objects are split into subgroups, group 1: (16, 13, 6, 20, 18, 17, 15, 8, 7, 10, 11) and group 2: (2, 4, 1, 14, 19, 5, 12, 3, 9), mean yields obtained for these subgroups will be 8.419 and 7.549 respectively (Table 2). The F test (Table 3) confirms significance of differences between subgroups in terms of mean yields ($F_{emp} = 18.2058$), whereas differences of mean yields obtained for objects within each subgroup are not significant ($F_{emp} = 0.3927$).

This example proved that the analysis of variance cannot give fully satisfactory results of multiple mean comparisons. This happens because ANOVA is based on comparison of all possible independent differences between pairs of means. If the analysis concerns of many small differences and only few large ones then global null hypothesis cannot be rejected, because sum of squares which measures these differences is too small relative to degrees of freedom corresponding to a number of comparisons. Such situation may occur quite frequently in practice, therefore modification of ANOVA technique to obtain homogeneous subgroups is justified. It should be noted that the analysis of variance described above (a comparison of two subgroups of analyzed objects) is nothing but a comparison known as a contrast between effects of tested objects.

Analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	19	11.6468	0.6130	0.8830	0.6039
Objects	19	17.5452	0.9234	1.3303	0.2175
Residual	41	28.4613	0.6942		

Table 2

Postulated division into groups

Group I												
Object	16	13	6	20	18	17	15	8	7	10	11	\bar{y}_I
Mean	9.057	8.794	8.744	8.662	8.648	8.489	8.322	8.054	8.013	7.917	7.904	8.419
Group II												
Object	2	4	1	14	19	5	12	3	9			\bar{y}_{II}
Mean	7.796	7.701	7.611	7.597	7.470	7.458	7.454	7.453	7.399			7.549

Table 3

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	19	11.6468	0.6130	0.8830	0.60394
Objects	19	17.5452	0.9234	1.3303	0.21754
<i>including:</i>					
Between groups	1	12.6380	12.6380	18.2058	0.00011
Within groups	18	4.9072	0.2726	0.3927	0.98235
Residual	41	28.4613	0.6942		

ORTHOGONAL CONTRAST CONSTRUCTION

Consider a modified technique of analysis of variance for the model (1): randomized complete one-factor design. The hypothesis (2) for this design is that all mean values calculated for tested objects represent one homogeneous group centered around the estimated experimental mean (m).

Rephrase our problem as follows: there are subgroups of examined objects having estimated mean values centered around the subgroup mean (subgroup centroid) similar as in the model (1) all object means are centered around an overall mean. One can always guess the existence of such subgroups but they must be properly

identified to ensure rejecting the null hypothesis of equality between subgroup means (centroids) without rejecting the null hypothesis of differences between means within these subgroups.

We will use one of hierarchical cluster analysis methods known as centroid clustering to identify such object subgroups. In centroid clustering distance between two clusters is defined as distance between their centers of gravity (here: between means/average point in the multidimensional space defined by values of analyzed variables). Agglomeration procedure assumes that each object creates initially a separate cluster. Assuming that there is at most p object subgroups, agglomeration procedure results in subsequent divisions into separate subgroups of objects as number of subgroups is reduced from $p-1$ to 2 based on an arbitrary distance measure, for example Euclidean or square Euclidean distance between means of each subgroup. Distances of Student or Fisher which take into account experimental design may be also used (Ludański, 1996; Mańkowski, 2002). The Fisher distance may be expressed as:

$$Q = \frac{n_s n_t (\bar{y}_s - \bar{y}_t)^2}{n_s + n_t} = n_s \bar{y}_s^2 + n_t \bar{y}_t^2 - \frac{(n_s \bar{y}_s + n_t \bar{y}_t)^2}{n_s + n_t} \quad (4)$$

whereas Student distance may be determined as:

$$\sqrt{Q} = \sqrt{\frac{n_s n_t (\bar{y}_s - \bar{y}_t)^2}{n_s + n_t}} \quad (5)$$

In both formulas s and t are indicators of subgroup means \bar{y}_s and \bar{y}_t respectively, n_s and n_t denote numbers of observations that correspond to subgroup means and are combined into one subgroup containing $n_s + n_t$ observations while reducing the number of subgroups from $(v + 1)$ to v . Note that the formula (4) expresses sum of the squares of contrast between groups identified with subscripts s and t . In analysis of variance contrast is defined as linear function of object means of known constant coefficients sum of which is equal to 0. In other words, if vector $\mathbf{c} = [c_1, c_2, \dots, c_p]'$, where $\sum_{s=1}^p c_s = 0$, expresses estimated contrast (comparison) between means (components of vector \mathbf{a}) established as $\mathbf{c}'\mathbf{a}$ then sum of squares calculated for the following hypothesis:

$$H_0: \mathbf{c}'\mathbf{a} = 0 \quad (6)$$

is equal to

$$Q = \mathbf{a}'\mathbf{c}[\mathbf{c}'\mathbf{C}\mathbf{c}]^{-1}\mathbf{c}'\mathbf{a} \quad (7)$$

where \mathbf{C} is a matrix such that the covariance matrix of vector \mathbf{a} is equal to $S_e^2\mathbf{C}$.

In particular case, if $c_s = 1$ and $c_t = -1$ while other coefficients are zero, then Q expresses the sum of squares of F statistic which tests equality of means calculated for subgroups s and t . Mindful of the relationship $t_{\alpha;n-p}^2 = F_{\alpha,1,n-p}$ one may apply Student's t statistic instead of F statistic to test hypothesis expressed by formula (6). Note that Student's test may be one-sided, i.e. may verify hypothesis written as:

$$H_0: c'a \leq 0 \tag{8}$$

If so, then testing statistic takes the form of $t_{emp} = \sqrt{\frac{Q}{s_e^2}}$ and consequently the null hypothesis formulated in equation (8) should be rejected on the significance level α , if $t_{emp} > t_{2\alpha,n-p}$ or by analogy if $F_{emp} = \frac{Q}{s_e^2} > F_{2\alpha,1,n-p}$.

Example 2

Consider data from Table 4 to introduce procedure described above. Table 4 presents ANOVA results obtained for experiment carried out for 5 corn cultivars. The experiment was performed in completely random design with 6 replications. Corn yield expressed in kilograms per plot (experimental unit) was a dependent variable. Mean yields per plot are presented in Table 5. Assuming the existence of 5 subgroups (each cultivar corresponds to separate subgroup) matrices of Fisher distances (tab. 6) between subgroup means may be determined according to the formula (4). As a final result two subgroups (Tab. 7) are obtained with Fisher distance equal to:

$$Q = \frac{24 \cdot 6 \cdot (99.4 - 71.75)^2}{24 + 6} = 3669.708 \tag{9}$$

Table 4

Analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Objects	4	5267.9284	1316.9821	190.3427	2.49E-18
Residual	25	172.9751	6.9190		

Table 5

Object means values ($n_i = 6$)

Objects	5	2	1	4	3	\bar{y}
Means	107.75	105.65	97.15	87.05	71.75	93.87

Table 6

Matrix of Fisher distances

<i>Step I</i>				
Object	2	1	4	3
5	13.23	337.08	1285.47	3888.00
2	×	216.75	1037.88	3447.63
1	×	×	306.03	1935.48
4	×	×	×	702.27

<i>Step II</i>			
Object	1	4	3
5,2	364.81	1544.49	4886.01
1	×	306.03	1935.48
4	×	×	702.27

<i>Step III</i>		
Object	1,4	3
5,2	1278.96	4886.01
1,4	×	1656.49

Table 7

Mean values of groups

Groups	(1,2,4,5)	(3)	\bar{y}
Means	99.40	71.75	93.87
n_i	24	6	30

Results presented in extended ANOVA table (tab. 8) for this experiment show that 5 corn cultivars form 4 homogeneous groups. Mean yields calculated separately for cultivar 5 and 2 do not differ significantly between each other, while mean yield representing these two cultivars differs significantly from mean yields observed in other three cultivars each of which forms a separate homogeneous group. Table 9 presents a series of contrasts which exhausts the set of all possible orthogonal contrasts available for this experiment. Fisher distance corresponds to the sum of squares calculated for the contrast of compared objects. Different distance measures eg. Euclidean distance allows to obtain the same or a different set of orthogonal

contrasts. For example, formula (9) for computing the square Euclidean distance takes the following form:

$$Q = (99.4 - 71.75)^2 = 764.5225. \quad (10)$$

Table 8

Complex/extended analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Objects	4	5267.928	1316.982	190.343	2.49E-18
H_0 : Test subjects do not form a homogeneous groups					
2 groups	1	3669.708	3669.708	530.381	2.37E-18
3 groups	1	1278.960	1278.960	184.847	4.73E-13
4 groups	1	306.0301	306.0301	44.230	5.72E-07
5 groups	1	13.230	13.230	1.9121	0.179
Residual	25	172.975	6.919		

Table 9

Set of orthogonal contrasts

Objects	1	2	3	4	5	SS
Contrast 1	0	-1	0	0	1	13.230
Contrast 2	1	0	0	-1	0	306.030
Contrast 3	1	-1	0	1	-1	1278.960
Contrast 4	1	1	-4	1	1	3669.708

Example 3

Consider experiment conducted in randomized complete blocks where effect of corn cultivar on yield per plot was studied. Results of ANOVA for experimental data, extended by orthogonal contrasts, are presented in Table 10. The analysis showed that 8 corn varieties formed 4 homogeneous groups regarding mean yield per plot. Mean yields calculated for each cultivar, homogeneous groups obtained according to proposed method and well known multiple comparison procedures are summarized in Table 11. It should be noted that standard multiple comparison procedures resulted in inseparable homogeneous groups. Complete separation of homogeneous groups is rarely attainable in practice, particularly if a large number of analyzed objects (means) is taken into account. The application of orthogonal contrasts enables complete separation of homogeneous groups (mutually independent) in each case.

Table 10

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	2	5.643	2.821	0.0595	0.94247
Cultivars	7	2347.247	335.321	7.0776	0.00099
Test subjects do not form a homogeneous groups					
2 groups	1	1476.056	1476.056	31.1549	0.00007
3 groups	1	601.142	601.142	12.6882	0.00313
4 groups	1	223.414	223.414	4.7156	0.04757
5 groups	1	43.867	43.867	0.9259	0.35226
6 groups	1	2.160	2.160	0.0456	0.83398
7 groups	1	0.327	0.327	0.0069	0.93497
8 groups	1	0.282	0.282	0.0059	0.93986
Residual	14	663.291	47.378		

Table 11

Homogeneous groups

Cultivar	\bar{y}_i	Orthogonal contrasts method	Tukey	Newman-Keuls	Duncan	Bonferroni	Scheffe	Student
1	104,87	a	a	a	a	a	a	a
4	104,40	a	ab	ab	ab	ab	ab	ab
8	94,43	b	abc	abc	b	abc	ab	b
6	93,23	b	abc	abc	b	abc	ab	b
2	87,73	c	abc	bc	bc	abc	ab	bc
7	87,30	c	bc	bc	bc	abc	ab	bc
3	82,83	c	c	c	c	bc	b	c
5	73,60	d	c	c	c	c	b	c

Example 4

An experiment discussed by Wagner (1977) will be used to present direct comparison of method based on minimal orthogonal contrasts with procedure based on minimal within-group sum of squares. The experiment concerned 14 cultivars of sugar beet and was realized in completely randomized block design with 6 replications. Sugar yield was a dependent variable and mean yields (dt/ha) obtained for each cultivar are presented in Table 12. Table 13 presents results of ANOVA,

extended by orthogonal contrasts for experimental data. Comparison procedure based on minimum within-group sum of squares resulted in 3 homogeneous groups of object means, while application of Tukey procedure allowed to obtain 2 homogeneous groups (Wagner, 1977). Method of minimal orthogonal contrasts based on Fisher distance between object means resulted in distinguishing 4 homogeneous groups (Tab. 14). Thus the most numerous group discussed by Wagner (1977) had been split into 2 separate subgroups (group 1 and 2 in Tab. 15). It is not difficult to note that the application of orthogonal contrasts resulted in considerable span of mean sugar yields observed between groups, whereas within each group object means calculated for cultivars were concentrated around group mean.

Table 12

Mean yield values of compared beet cultivars

Cultiv.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Yield	98.36	100.87	107.58	102.32	105.11	106.13	102.03	102.29	100.58	84.11	95.52	101.91	96.44	103.05

Table 13

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	5	193.05	38.61	2.175	0.067615
Cultivars	13	2610.79	200.83	11.314	3.53E-12
H_0 : Test subjects do not form a homogeneous groups					
2 groups	1	1725.174	1725.174	97.193	1.56E-14
3 groups	1	569.553	569.553	32.087	3.62E-07
4 groups	1	244.935	244.935	13.799	0.000425
5 groups	1	22.658	22.658	1.276	0.262796
Within groups	9	48.470	5.386	0.303	0.971258
Residual	65	1153.75	17.75		

CONCLUSIONS

The alternative multiple comparison procedure incorporating orthogonal contrasts to determine homogeneous groups of objects undergone analysis of variance enables complete separation of analyzed object means that means within homogeneous groups do not differ significantly between each other but between-group means (centroids) are significantly different. Moreover, significant association of group variation relative to the total object variation ensures optimal separation of object means into distinct homogeneous groups. Proposed procedure may be applied for each linear ANOVA model and analysis of covariance of classified data.

Commonly used multiple comparison procedures are based generally on comparing the distances between means calculated for pairs of objects relative to the appropriate error that results from covariance matrix of these means (thus they correspond to the matrix of experimental design). Although these procedures are very useful for comparison selected objects to each other (answering the question: does cultivar A differ significantly from cultivar B in terms of mean value of studied feature) applying them to split objects into homogeneous subgroups results in an approximate picture of possible separation, especially if number of objects is large. The procedure discussed in this paper consists in determination of orthogonal contrasts between means according to the criterion of minimum contrast and it seems to meet the expectations of practitioners.

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